1(Fourth amended). An assay method for the determination of holo-transcobalamin II (holo-TC II)in a body sample, comprising contacting a cell free sample of a body fluid with an immobilised or immobilizable specific binding ligand for holo-transcobalamin II (holo-TC II), whereby to form bound holo-TCII, separating a ligand bound fraction from a non-ligand bound fraction, releasing said bound holo-TCII from the ligand bound fraction into a volume of liquid so reduced in comparison with the volume of said cell free sample, to provide a cobalamin containing liquid having a cobalamin concentration at least 3 times the holo-TCII concentration in said cell free sample, and determining the holo-TCII content in said body sample by measuring the amount of cobalamin or TCII-protein in said cobalamin containing liquid arising from the bound holo-TCII released from the specific binding ligand and relating this to the volumes of said cobalamin containing liquid and said cell free sample.

D2

3(Amended). An assay method as claimed in claim 1 or claim 51 wherein said assay is capable of detecting holo-TC II in said body sample at a concentration as low as 9 pM.

D3

4(Thrice Amended). An assay method as claimed in claim 1 or claim 51 wherein said assay is effected to analysis by an automated process.

5(Twice Amended). An assay method as claimed in claim 1 or claim 51 wherein said specific binding ligand is selected from the group consisting of polyclonal and monoclonal antibodies, antibody fragments, polypeptides, oligonucleotides, small organic chemicals, specific binders selected from combinatorial chemistry libraries, specific binders selected from phage display libraries, specifically binding sequences of DNA, and specifically binding sequences of RNA.

D4

7(Amended). An assay method as claimed in claim 1 wherein said specific binding ligand exhibits a high degree of selectivity towards holo-TCII and exhibits low affinity towards other cobalamin binding proteins, in either apo or holo form.

D5

9(Twice Amended). An assay method as claimed in claim 1 or claim 51 wherein said bound holo-TCII is released by changing the temperature or the pH of the surrounding medium.

10(Amended). An assay method as claimed in claim 1 or claim 51 wherein the different cobalamin forms are converted to the less light sensitive cyanocobalamin by treatment with KCN prior to contacting said sample with a specific binding ligand.

D6

11(Amended). An assay method as claimed in claim 1 or claim 51 wherein the cobalamin content of said cobalamin containing liquid is measured by a competition assay performed by contacting an immobilised binding partner for cobalamin with the dissociated cobalamin of the sample in the presence of labelled ligand which competes with the isolated cobalamin for binding to the immobilised binding partners.

12(Amended). An assay method as claimed in claim 51 wherein the binding ligands for TC II are immobilised and bind to both holo- and apo-TC II.

16(Amended). An assay method as claimed in claim 51 wherein a preliminary separation step is carried out using an immobilised or immobilisable cobalamin or an analogue or fragment thereof which selectively binds the apo-forms of both TC II and haptocorrin (HC) contained within said cell free sample, such that the apo forms of the TC II and HC proteins are bound by the cobalamin, analogue or fragment thereof and separated from the holo-TC II and holo-HC complexes, wherein said preliminary step is carried out prior to contacting said cell free sample with said specific binding ligand.

D7

Please delete claims 19 and 24 without prejudice or disclaimer.

25(Twice Amended). An assay method as claimed in claim 1 wherein at least 80% of holo-TC II present within said cell free sample is contained within said ligand bound fraction.

D8

26(Twice Amended). An assay method as claimed in claim 1 or claim 51 further comprising a preliminary separation step in which the cell free sample is contacted with an immobilized or immobilizable specific binding ligand for haptocorrin wherein said preliminary step is carried out prior to contacting said cell free sample with said specific binding ligand.

D8

27(Amended). An assay method as claimed in claim 51 wherein said TC II binding ligand possesses an affinity constant of at least 10^9M^{-1}.

D9

31(Amended). An assay method as claimed in claim 1 or claim 51 wherein the degree of cross reactivity of said specific binding ligand with HC is less than 1%.

35(Twice Amended). An assay method as claimed in claim 1 or claim 51 wherein the concentration of cobalamin in said cobalamin containing liquid is at least 5-fold greater than the concentration of cobalamin in said sample.

D10

36(Amended). An assay method as claimed in claim 1 or claim 51 wherein the concentration of cobalamin in said cobalamin containing liquid is at least 10-fold greater than the concentration of cobalamin in said sample.

42(Twice Amended). An assay method as claimed in claim 1 or claim 51 wherein said body sample is selected from the group consisting of seminal fluid, cerebro-spinal fluid, amniotic fluid and blood derived samples.

D11

44(Amended). An assay method as claimed in claim 1 or claim 51 wherein said bound fraction is separated from said unbound fraction by precipitation, centrifugation, filtration or chromatographic methods.

D12

47(Amended). An assay method as claimed in claim 1 or claim 51 wherein said specific binding ligands are immobilised on a particulate solid phase support.

D13

49(Twice Amended). A kit for use in a diagnostic assay according to claim 1 or claim 51, comprising:

an immobilized or immobilizable specific binding ligand for TC II or holo-TC II;

a plurality of holo-TC II solutions of known concentration;

a release agent to release cobalamin from holo-TC; and

optionally a labelled ligand

50(Amended). An assay method as claimed in claim 1 or claim 51 wherein the cobalamin content in said cobalamin containing liquid is determined in a competitive binding assay.

Please add the following new claims to the application.

51(New). An assay method for the determination of holo-transcobalamin II (holo-TC II)in a body sample, comprising contacting a cell free sample of a body fluid with an immobilised or immobilizable specific binding ligand for transcobalamin II (TC II), whereby to form bound TCII consisting of bound holo-TCII and bound apo-TCII, separating a ligand bound fraction from a non-ligand bound fraction, releasing said bound TCII from the ligand bound fraction into a volume of liquid so reduced in comparison with the volume of said cell free sample, to provide a cobalamin containing liquid having a cobalamin concentration at least 3 times the holo-TC II concentration in said cell free sample, and determining the holo-TCII content in said body sample by measuring the amount of cobalamin or TCII-protein in said cobalamin containing liquid arising from the bound holo-TCII released from the specific binding ligand and relating this to the volumes of said cobalamin containing liquid and said cell free sample.

52(New). An assay method as claimed in claim 51 wherein said specific binding ligand exhibits a high degree of selectivity towards TCII and exhibits low affinity towards other cobalamin binding proteins, in either apo or holo form.

D 16

53(New). An assay method as claimed in claim 1 wherein at least 80% of TC II present within said sample is contained within said ligand bound fraction.